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PASTURE AND FORAGE UTILIZATION

Evaluation of sugarcane silages added with macaúba cake

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ABSTRACT. This study evaluated the pH and contents of dry matter (DM) and lactic acid (AL) of sugarcane silages added with different levels of inclusion of macaúba cake (TM) in different opening times (TA), in days. Laboratory silos used were opened at times 0; 3; 7; 12; 24; 36; 41 and 60 days after ensiling. The response surface methodology was used for statistical analysis. The DM content was influenced by both NI and TA. The inclusion of TM contributed to increase the DM content of sugarcane, as it absorbed moisture of the ensiled sugarcane. The pH values were influenced only by TA, which were lower than 3.8. The AL content was influenced by both NI and TA, that is, the inclusion of TM favored the increase of AL content over the 60 days evaluated. It can be concluded that the inclusion of TM in sugarcane silage can be used in all NI evaluated, and the silages should be stored for 60 days to ensure the adequate fermentation process inside the silos.

Keywords: lactic acid; opening times; response surface.

Avaliação de silagens de cana de açúcar adicionadas de torta de macaúba

RESUMO. Analisaram-se os teores de matéria seca (MS), os valores de pH e os teores de ácido lático (AL) de silagens de cana de açúcar adicionadas de diferentes níveis de inclusão (NI) de torta de macaúba (TM) em diferentes tempos de abertura (TA), em dias. Utilizou-se silos laboratoriais que foram abertos nos tempos 0; 3; 7; 12; 24; 36; 41 e 60 dias após a ensilagem. Para a análise estatística, utilizou-se a metodologia de superfície de resposta. Os teores de MS foram influenciados tanto pelo NI quanto pelo TA. A inclusão da TM contribuiu para aumentar os teores de MS da cana de açúcar, pois atuou como absorvente de umidade da cana ensilada. Os valores de pH foram influenciados apenas pelo TA, que foram inferiores a 3,8. Os teores de AL foram influenciados tanto pelo NI quanto pelo TA, ou seja, a inclusão da TM favoreceu o aumento dos teores de AL ao longo dos 60 dias avaliados. Conclui-se que a inclusão da TM à ensilagem de cana de açúcar pode ser utilizada em todos os NI avaliados, e as silagens devem ser armazenadas por 60 dias, para garantir o adequado processo fermentativo dentro dos silos.

Palavras-chave: ácido lático; superfície de resposta; tempos de abertura.

Introduction

The global concern about the environmental issue, along with the search for renewable energy sources, places biofuel at the center of attention and interest. The production of biofuel is obtained through transesterification, with separation of glycerin and vegetable oil. There are the generation of two products: esters and glycerin (widely used in soap); and the main co-products generated are cake and meal, which can be used for other purposes, thus constituting other sources of income that are important for producers (Abdalla, Silva Filho, Godoi, Carmo, & Eduardo, 2008).

According to Teixeira et al. (2014), these co-products originate from the extraction of oil from oilseed crops, reducing the cost of production of both biofuel and animal feed if this is the destination of these co-products. However, if they are not properly disposed, they can cause various environmental problems, such as pollution and environmental contamination, as well as wasting foods with high nutritional value.

Tropical forages show a marked seasonality, determined by the uneven distribution of water throughout the year, with forage remains in the rainy season and lack in the dry season, thus generating an impairment of performance of the animal production. The adoption of forage conservation techniques that preserve the nutritional characteristics and allow the use during critical periods of the year becomes extremely necessary, standing out the ensiling technique.

Sugarcane has the main characteristics necessary for silage production, such as: dry matter content around 30%, soluble carbohydrate content above 10% of natural matter and buffering power, which allows the pH drop to values close to 3.5, due to the production of organic acids, such as lactic acid, for example. However, sugarcane silage presents some obstacles, and the supply of additives is necessary (Freitas et al., 2006).

The addition of a co-product with high dry matter content and good nutritive value functions as an absorbent additive, since sugarcane contains 70 to 80% moisture, raising the dry matter content of the ensiled material and making the medium less favorable to the development of yeasts. Under aerobic conditions, many species of yeast degrade lactic acid into CO_2 and H_2O. Degradation of lactic acid causes a rise in the pH of the silage, leading to the growth of other microorganisms capable of deteriorating the silage at a higher pH (McDonald, Henderson, & Heron, 1991).

The study of the potential use of co-products generated with the production of biofuels and their use in animal feed makes it possible to remove potential environmental impact factors from the environment and, together, allows to obtain better quality forages for ruminant feed.

Given the above, this study aimed to evaluate the dry matter (DM) content, pH values and lactic acid (LA) content of silages of sugarcane (*Saccharum* spp.) added with different levels of macaúba cake, in different opening times, in days, in order to determine the quality of the silages according to the aforementioned parameters.

Material and methods

The experiment was conducted at the Federal University of Jequitinhonha and Mucuri Valleys (UFVJM), Campus JK, located in the municipality of Diamantina, State of Minas Gerais, Brazil. The analyses were carried out in the Biofuels Laboratory and the Animal Nutrition Laboratory, both at UFVJM.

Different levels of inclusion of the co-product from biofuel production, macaúba cake, in sugarcane silage were studied, at the following proportions (NI): 0, 3, 10, 17 and 20%, in relation to the dry matter of sugarcane, and at different opening times (TA): 0, 3, 7, 12, 24, 36, 41 and 60 days after ensiling.

Sugarcane was obtained from the Experimental Farm of Moura (FEM) of UFVJM, located in the municipality of Curvelo, State of Minas Gerais, Brazil. Sugarcane was harvested at 10 cm from the ground and the whole plant was taken to Campus JK, where the material was chopped in a stationary machine adjusted to obtain particles of approximately 2 cm. Macaúba cake (pulp and peel) was supplied by DBIO Company, located in Dores do Indaiá, State of Minas Gerais, Brazil. Macaúba cake was also chopped in the machine in order to obtain homogeneous particles for ensiling.

After the addition of the co-product to the chopped sugarcane in the described proportions, the material was homogenized and ensiled in laboratory silos made of PVC tubes of 100 mm diameter and 450 mm in length, and the compaction was done using wooden sockets. The material was ensiled to a density of 600 kg m^{-3}. The silos were sealed at the time of ensiling with PVC caps fitted with Bunsen valves to allow the gases generated during the fermentation process into the silos to escape and then sealed with adhesive tape.

The silos were opened in the aforementioned opening times. Upon opening, at all opening times, the material was homogenized and a part of the contents of each silo was pressed with a hydraulic press for extracting the juice from the silage, from which a part was used to determine pH values using an expanded scale potentiometer, Tecnopon mPA 210. A certain volume of the silage juice was used for the analysis of organic acids, more precisely lactic acid, using a High-Performance Liquid Chromatograph (HPLC). The identification and quantification of lactic acid was performed using the C18 Bio-Rad column (Reverse Phase). The sugarcane presented 0.68 dag kg^{-1} lactic acid at the time of ensiling.

Another part of the contents of the silos was pre-dried in a forced ventilation oven, and the samples were weighed inside paper bags and taken to the oven at 55ºC for 72 hours, according to the methodology described by Silva and Queiroz (2002). Samples were then weighed and milled in a Willey stationary mill with a 1 mm sieve (Association of Official Analytical Chemist [AOAC], 2005) and packed in plastic bags for further determination of dry matter contents. The dry matter (DM) content of sugarcane and macaúba cake, at ensiling, were 25.1 and 84.8%, respectively.

For the statistical analysis, data were evaluated through the response surface methodology, using Statistica 7.0 software. The response surface methodology is a statistical technique used for modeling and analysis of problems in which the variable response is influenced by several factors, whose objective is the optimization of this response. Therefore, for the study in question, the variables studied can be influenced both by the different levels of inclusion of the cake, and by the different opening times, in days, of the silos. Thus, the response surface methodology allows a broader view of the variables under study with all the factors involved (Marone et al., 2015).

For planning the experiment, the desired factors were studied so that there was a pre-planning before implementing it in the Statistic software. After this step, the program generated the planning, which was based on the independent factors, which are the inclusion levels and the opening times (in days). The response variables were the contents of DM, pH values and contents of lactic acid. Thus, for data analysis 36 silos were used in total.

Results and discussion

We tested the hypothesis of influence of the different inclusion levels (NI) of the macaúba co-product and the different opening times (TA), in days, as well as their interactions, on the dry matter (DM) content of sugarcane silages. Considering 5% significance, DM contents were influenced by both TA and NI, but were not influenced by the interaction of these two factors.

Data of the statistical analysis, p-value, coefficient of variation (CV) and coefficient of determination (R^2) obtained for the DM content are listed in Table 1.

Table 1. Statistical parameters of the response variable related to the dry matter content (%DM) of sugarcane silages added with different inclusion levels (NI) of macaúba cake and evaluated at different opening times (TA), in days.

Response variable	Independent Factors	Behavior	P	CV	R^2
	TA	Linear	0.0028*	3.5591	
	TA	Quadratic	0.0594*	3.5199	
%DM	NI	Linear	0.0005*	5.8735	0,6737
	NI	Quadratic	0.6917^{NS}	0.4585	
	TAXNI	-	0.3358^{NS}	1.2164	

P – statistical model significance level; CV – coefficient of variation; R^2 - coefficient of determination of the equation; *significant model ($p < 0.05$); NS – non-significant model.

Through statistical analysis, we obtained a simplified descriptive model for the behavior of the response variable (DM content):

Equation 1:

$$\%MS = 26.2124 + 1.0277 * NI - 0.0016 * TA$$

where: %DM = dry matter content; NI = inclusion level; TA = opening time.

Using Equation 1, the response surface of the dry matter content was generated as a function of the inclusion levels of the macaúba cake and the opening times of the silos, in days, which is shown in Figure 1.

According to the statistical analysis, it was observed that the TM contributed to increase the DM content of sugarcane silages, since the independent factor NI presented increasing linear behavior, which favored the increase in DM contents.

The fresh sugarcane presented 25.10% DM, and the TM, 84.79%. The co-product was added to the silage to raise the DM content of the ensiled material, contributing to the fermentation process inside the silos and increase the nutritional value of the silages produced.

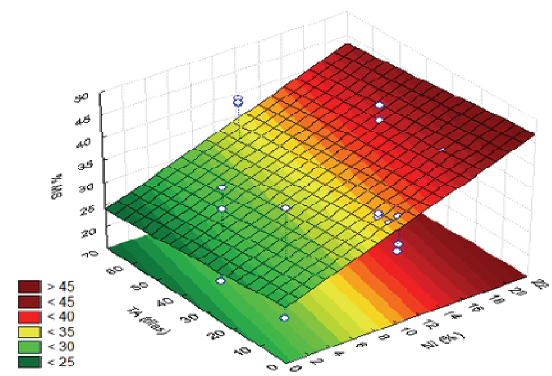

Figure 1. Response surface related to the behavior of dry matter content (%DM). Behavior presented from the simplified model (Equation 1). DM = Dry matter; TA = Opening time, in days; NI = Inclusion level.

In the silages prepared exclusively with sugarcane, the DM contents did not change over the evaluated time, that is, at T = 0, the silages presented approximately 26% DM. After the 60 days evaluated, the sugarcane silages presented DM contents close to the original material (26%).

With the inclusion of 3% TM in sugarcane silage, DM contents of silages increased to 29.2%, and remained in this range throughout the fermentation process inside the silos, evidencing the stability of this variable along of the evaluated time. The inclusion of TM was responsible for these increases in DM content of silages, since the macaúba cake absorbed the moisture of sugarcane silage. It was observed the same behavior for the other inclusion levels of TM, since the DM contents of the silages reached levels of 36.5, 43.7 and 46.8%, when 10, 17 and 20% TM, respectively, were added to the silage.

The low dry matter content found in sugarcane and silage composed of exclusively sugarcane has been pointed out as one of the main limitations for the production of quality silages. It should be considered that the high moisture content of ensiled forage results in excessive effluent production, which not only hinders handling but also carries highly digestible nutrients, which decreases the nutritive value of the silages and favors the increase of proteolysis in the ensiled material and consequently, the establishment of clostridial bacteria (Oude-Elferink, Driehuis, Gottschal, & Spoelstra, 2000).

Therefore, the evaluated macaúba co-product contributed in fact to raise the DM contents of the ensiled sugarcane, which can be observed in Figure 1, where the white points are the treatments evaluated and the response surface is the optimization of the data obtained. That is, it is a projection of the behavior of this variable with reference to the initial statistical planning and the data effectively studied.

As for the pH values, we tested the influence of the different inclusion levels (NI) of the macaúba co-product and the different opening times (TA) on the pH values of the sugarcane silages, as well as their interactions. Considering 5% significance, the pH values were influenced only by the TA of the silos, in days.

Data of the statistical analysis, p-value, coefficient of variation (CV) and coefficient of determination (R^2) obtained for pH values are listed in Table 2.

Table 2. Statistical parameters of the response variable related to the values of pH of sugarcane silages added with different inclusion levels (NI) of macaúba cake and evaluated at different opening times (TA), in days.

Response Variable	Independent Factors	Behavior	P	CV	R^2
	TA	Linear	0.0955^{NS}	0.0001	
	TA	Quadratic	0.0046*	0.7810	
pH	NI	Linear	0.3547^{NS}	0.2038	0.4714
	NI	Quadratic	0.2595^{NS}	0.0001	
	TAXNI	-	0.7988^{NS}	0.1601	

P – statistical model significance level; CV – coefficient of variation; R^2 - coefficient of determination of the equation; *significant model ($p<0.05$); NS – non-significant model.

Through statistical analysis, we obtained a simplified descriptive model for the behavior of the response variable (pH):

Equation 2:

$$pH = 3.8832 - 0.0668 * TA + 0.0009 * TA^2$$

where: pH = potential of hydrogen; TA = opening time; NI = inclusion level.

The linear factor of TA, as well as the factors of NI and the interaction between TA and NI do not affect the behavior of pH, at 5% of significance. However, according to Van Soest (1994), during the fermentation process that takes place inside the silos, there is organic acid production, which is also responsible for the reduction in pH values. Therefore, the acceptance of the hypothesis H_0 for the linear factor of TA can cause the Type II error. Thus, the linear factor of TA was considered in the equation that describes the behavior of pH values (Equation 2).

By means of Equation 2, the response surface of pH was generated according to the inclusion levels of the macaúba cake and the opening times of the silos, in days, illustrated in Figure 2.

For all NI of TM, the pH values remained the same as the mean, i.e., equal to 3.8 (Figure 2). However, when the TA was evaluated, it was observed that the pH values were lower than 3.8, for all opening times, which complies with the recommendation of McDonald (1981), who suggests a pH equal to or less than 3.8 units to obtain good fermentation of the ensiled material and consequently, good preservation of this material. In addition, Valeriano et al. (2009) reported that the rapid fall in pH of sugarcane silages prevents the growth of microorganisms that degrade proteins, such as enterobacteria and clostridia, which are microorganisms responsible for the secondary and undesirable fermentations in the ensiled material.

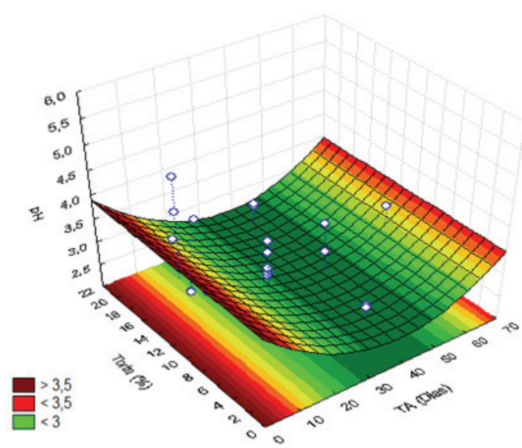

Figure 2. Response surface related to the behavior of pH. Behavior presented from the simplified model (Equation 2). pH = potential of hydrogen; Torta = inclusion level of macaúba cake; TA = Opening time, in days.

It was also verified in Figure 2 that, from the 36th day of silage storage, the pH values were constant, remaining equal to 2.6 until the 48th day of storage, evidencing that there was stabilization of fermentation during this period. This stage of stability of the fermentation remains as long as it is anaerobic, which is responsible for keeping preserved the ensiled material (Oude-Elferink et al., 2000). From day 49, the pH values of the silages gradually increased, reaching values equal to 3.0, when the silages were stored for 60 days (TA = 60).

In relation to the lactic acid content, we tested the influence of the different inclusion levels of the macaúba co-product and the different opening times (TA), in days, as well as their interactions, on the production of lactic acid of sugarcane silages. Considering 5% significance, the lactic acid contents were influenced by both TA and NI, but were not influenced by the interaction of these.

Data of the statistical analysis, p-value, coefficient of variation (CV) and coefficient of determination (R^2) obtained for lactic acid contents are presented in Table 3.

Table 3. Statistical parameters of the response variable related to the lactic acid content (%AL) of sugarcane silages added with different inclusion levels (NI) of macaúba cake and evaluated at different opening times (TA), in days.

Response Variable	Independent Factors	Behavior	P	CV	R^2
	TA	Linear	0.0001*	0.6453	
	TA	Quadratic	0.0045*	0.5664	
%AL	NI	Linear	0.6194^{NS}	0.0781	0.7252
	NI	Quadratic	0.0271*	0.4624	
	TAXNI	-	0.2001^{NS}	0.5894	

P – statistical model significance level; CV – coefficient of variation; R^2 - coefficient of determination of the equation; *significant model ($p<0.05$); NS – non-significant model.

Through statistical analysis, we obtained a simplified descriptive model for the behavior of the lactic acid content (%AL)

(Equation 3):

$$\%AL = 0.4370 + 0.0047 * NI^2 + 0.0789 * TA - 0.0006 * TA^2$$

in which: %AL = lactic acid content; TA = opening time; NI = inclusion level.

Using equation 3, the response surface of the lactic acid content was generated, as a function of the inclusion levels of the macaúba cake and the opening times of the silos, in days, which is shown in Figure 3:

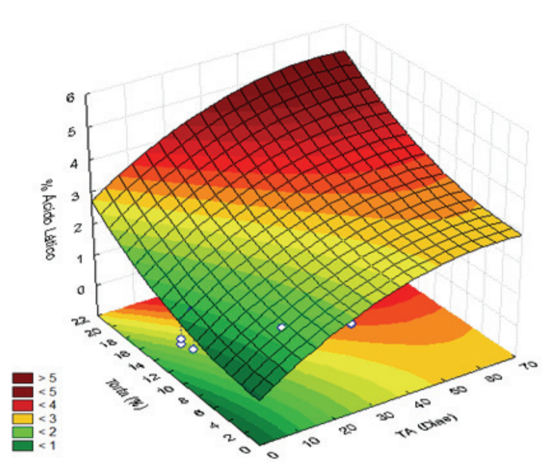

Figure 3. Response surface related to the behavior of lactic acid content (%AL). Behavior presented from the simplified model (Equation 3). Torta = inclusion level of macaúba cake; TA = Opening time, in days.

According to the statistical analysis, we verified that lactic acid (AL) contents increased with increasing NI of TM in the sugarcane silage, during the 60 days of silage storage. For the silages prepared exclusively with sugarcane, the contents of AL increased from 0.44 (T = 0) to 2.91% (T = 60). For the highest NI of TM (NI = 20%), the AL content increased from 2.32 (T = 0) to 4.79% (T = 60), evidencing the highest production of this organic acid with the highest NI and TA, which can be observed in Figure 3.

It is possible to infer that the AL production may have been responsible for the maintenance of pH stabilization (Figure 2) during the entire fermentation process inside the silos, probably due to the development of lactic acid bacteria. Obligatory homofermentative lactic acid bacteria produce more than 85% lactic acid from hexoses. Facultative heterofermentative bacteria also produce mainly lactic acid from hexoses, but are also capable of degrading some pentoses into lactic acid, acetic acid and/or ethanol (Devriese, Collins, & Wirth, 1992), which may justify the contents of lactic acid found in this work. Nevertheless, the lactic acid content verified in silages does not necessarily represent the total amount of this acid produced during the fermentation process, since part of it can be metabolized to ethanol by the yeasts present in the silage (Schmidt et al., 2011), although this process occurs more often under aerobiosis (McDonald et al., 1991.

The contents of AL found herein can be considered ideal for good quality silages, because, according to Vieira et al. (2004), silages with AL content between 2.0 and 3.0% can be considered as satisfactory silages.

Conclusion

The inclusion of all levels of macaúba co-product to sugarcane silage favored the fermentation process inside the silos, contributing to the rapid decrease in pH values and to the satisfactory production of lactic acid, promoting the good conservation of the ensiled material.

Regarding the storage time of the silages, these should be stored for at least 30 days, for all levels of inclusion evaluated. Shorter storage times do not guarantee an adequate fermentation process of the ensiled material, evidenced by the pH values that, during this period, presented a more pronounced drop than in the subsequent period.

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References

Abdalla, A. L., Silva Filho, J. C., Godoi, A. R., Carmo, C. A., & Eduardo, J. L. P. (2008). Utilização de subprodutos da indústria de biodiesel na alimentação de ruminantes. *Revista Brasileira de Zootecnia, 37*(Spe), 260-268.

Association of Official Analytical Chemist [AOAC]. (1995). *Official Methods of Analysis* (16th ed.). Arlington, VA: AOAC International.

Devriese, L. A., Collins, M. D., & Wirth, R. (1992). The genus enterococcus. In Balows, A., Trüper, H. G., Dworkin, M., Harder, H., & K. H. chleifer (Ed.), *The prokaryotes* (2nd ed.). New York, NY: Springer Verlag (p. 1465-1481).

Freitas, A. W. P., Pereira, J. C., Rocha, F. C., Detmann, E., Ribeiro, M. D., Costa, M. G., & Leonel, F. d. P. (2006). Características da silagem de cana de açúcar tratada com inoculante bacteriano e hidróxido de sódio e acrescida de resíduo da colheita de soja. *Revista Brasileira de Zootecnia, 35*(1), 48-59.

Marone, A., Varrone, C., Fiocchetti, F., Giussani, B., Izzo, G., Mentuccia, L., Rosa, S., & Signorini, A. (2015). Optimization of substrate composition for biohydrogen production from buffalo slurry co-fermented with cheese whey and crude glycerol, using microbial mixed culture. *International Journal of Hydrogen Energy, 40*(1), 209-218.

McDonald, I. (1981). A revised model for the estimation of protein degradability in the rumen. *The Journal of Agricultural Science, 96*(1), 251-252.

McDonald, P., Henderson, A. R., & Heron, S. J. (1991). *The Biochemistry of Silage* (Vol. 1). Aberyswyth, US: Chalcombe Publications.

Oude Elferink, S. J. W. H., Driehuis, F., Gottschal, J. C., & Spoelstra, S. F. (2000). Silage fermentation processes and their manipulation. *FAO Plant Production and Protection Papers, 1*, 17-30.

Schmidt, P., Rossi Junior, P., Junges, D., Dias, L. T., Almeida, R., & Mari, L. J. (2011). Novos aditivos microbianos na ensilagem da cana de açúcar: composição bromatológica, perdas fermentativas, componentes voláteis e estabilidade aeróbia. *Revista Brasileira de Zootecnia, 40*(3), 543-549.

Silva, D. J., & Queiroz, A. C. (2002). *Análise de alimentos: métodos químicos e biológicos* (3 ed.). Viçosa, MG: Universdiade Federal de Viçosa.

Teixeira, U. H. G., Simioni, T. A., Santos, P. D., Gomes, F. J., Paula, D. C., & Botini, L. A. (2014). Potencial de utilização de co-produtos agroindustriais para suplementos. *Revista Brasileira de Zootecnia, 40*(3), 543-549.

Valeriano, A. R., Pinto, J. C., Ávila, C. L. S., Evangelista, A. R., Tavares, V. B., & Schwan, R. F. (2009). Efeito da adição de *Lactobacillus* sp. na ensilagem de cana de açúcar. *Revista Brasileira de Zootecnia, 38*(6), 1009-1017.

Van Soest, P. J. (1994). *Nutritional ecology of the ruminant* (Vol. 1). Ithaca, NY: Cornell University Press.

Vieira, F. A. P., Borges, I., Stehling, C. A. V., Gonçalves, L. C., Coelho, S. G. C., Ferreira, M. C., & Rodrigues, J. A. S. (2004). Qualidade de silagens de sorgo com aditivos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 56*(6), 764-772.

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